Swami Ramanand Teerth Marathwada
University, Nanded, Maharashtra, India
(UGC recognised, NAAC Re-accredited 'B++' grade stateuniversity)

"Studies on Pesticide Residue Analysis of Leafy Vegetables and their Pest Management by Using Botanicals"

Research Proposal submitted
In partial fulfilment of registration to
Ph.D. Programme

In the subject
Botany under the faculty of Science and Technology

Submitted by

ANUJA DNYANESHWAR BOKHAREPATIL

Under the Supervision of
Dr. Saheb L. Shinde

Professor and Research Supervisor
Department of Botany,
Yeshwant Mahavidylaya,Nanded.
2023

Department of Botany, Yeshwant Mahavidylaya,Nanded

1. Introduction:

The demand of food is increasing daily worldwide, because of rapid growth of world population. To fulfill this demand, it is necessary to increase agricultural productivity. Farmers use the best combination of cultural, chemical and biological measures to keep crops pest free. According to the FAO(2017) India is a 2nd largest producer of a vegetables after china.

A Green leafy vegetable is considered as a good source of Vitamins, Minerals and Antioxidant. It also gives an integral component of a daily vegetarian diet. The majority of farmers who grow vegetables use pesticides to manage these pests because leafy vegetables are attacked by a wide variety of pests and diseases. Leafy vegetables are vital for a balanced diet and for providing variety of flavour to the daily meal. Pesticides are human made and harmful on the other hand Naturally occurring chemicals that controls insects. (xiao et al., 2010;Clarke et al., 1997). The pesticide contains of many compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators etc. Commonly used pesticides on green leafy vegetables are dichorvos (dcv), chlorpyrifos (cps), profenofos (pff), monocrotophos (mono), cypermethrin (cp) etc. in a very large quantity pests are found in a various crops to resist from this pests pesticidesare used mostly.

Each year, almost 2.5 million tonnes of pesticides are used globally, and this number is increasing. (FAO/WHO, 2002). However, these pesticides have a negative impact on both human health and the environment. The toxic chemicals dissolved in our water system and invaded the human ecology. Many animals and fish are harmful to their health.(Khandakar et al., 1990). Serious acute and chronic health impacts result from the widespread usage of these harmful substances. (Dammalas and Eleftherohorinnos, 2011). Human exposure to pesticide residues causes chronic health impacts such as hormone disruption, reproductive abnormalities, immunological suppression and cancer.

According to the Monitoring of Pesticide Residues at the National Level Annual Progress Report. (April 2013-March 2014). The Ministry of Agriculture's, Department of Agriculture and Cooperation began a central sector scheme, "Monitoring of Pesticide Residues at the National Level," in food commodities and environmental samples during 2005-06, with the participation of various laboratories representing the Ministry of Agriculture, The Indian Council of Agriculture Research. The scheme's major goals are to identify crops and regions with a high concentration of pesticide residues in order to focus extension efforts on Integrated Pest Management.

To avoid all the effects caused by the excessive use of pesticides, it is very necessary to find out about alternative pest management options. The biological method provides the best alternative to maintaining insect populations without causing harm to organisms or ecosystems. (LV et al., 2011). Botanicals are synthesised from fresh or dried plants, plant parts, or plants extracted in water and ethanol. These are naturally occurring plant products synthesised from plant materials. All researchers showed a general trend towards specific, bioactive, and safe molecules with minimum environmental hazards. Botanicals are an environmentally safe and rich source of biologically active compounds. The use of botanical pesticides plays a very important role in integrated pest management programmes. It has a wide range of behavioural and physiological effects on insects, and for insects, it is difficult to develop resistance against these botanical pesticides. Farmers can take up the formulation of locally available plants, and thus they will be saved from spending money on the purchase of costly synthetic pesticides. In an international market, there is a great demand for residue-free cotton, fruits, vegetables, and beverages. (kumbhar etal.,2020).

Botanical pesticides play a major role as they are easily and locally available and provide employment at the rural level. Botanicals have been used for pest control since the Vedic period. (Koul & Walia 2009). Over three lakh plant species are estimated to have pesticidal properties, but only 2400 species have been identified, and a lot more need to be exploited. (Thacker et al., 2002). Only 10% of the compounds present in the array of plant chemicals have been identified and due to this, a huge future scope for researchers is available in this field. To identify and find out about new botanical pesticides that are useful for maintaining pests and diseases on crops. It is also helpful to avoid the risk to human health, animals, fish, and the environment from pesticide residues and their adverse effects, so botanical pesticides are a good alternative for integrated pest management (IPM). These days, scientists are working on developing some new botanical pesticides that are very safe for humans.

2. Review Of Literature:

Pesticide application is one of the most important ways to protect crops from pests. Pesticide use, on the other hand, generates residual issues in vegetables and other food products. This is a big issue in various countries, including India. Pesticide residues are hazardous to species other than pests and illnesses. Pesticides have numerous negative repercussions on human life, wildlife, and aquatic life today.

Pesticides contribute significantly to the economies of developing countries, According to Ecobichonet et al. (2001). These developing countries are becoming key "breadbaskets" for the rest of the globe, capable of producing two or three crops per year. These objectives cannot be met

without the usage of pesticides. Pesticides such as insecticides, herbicides, and fungicides are not used in traditional agricultural practises. Pesticides were widely utilised for their beneficial effects on pests, weeds, fungus, and infections in the 1860s. Pesticides are typically used on agricultural pests to manage plant diseases, boost crop productivity, and reduce insect pests.

Torres et al. (1996) stated that using pesticides in agriculture provides many benefits to farmers, but it also sets back residues that remain in food. Due to the presence of residue, many health issues occur for consumers. Because of this, it is necessary to control their residue level via Fixing limits and emphasising products that are less toxic for human health. Chanda et al. (2014) reported that pesticides are compounds used to prevent organisms harmful to humans. Depending upon the types of pests controlled, toxic properties, and chemical composition, pesticides are classified into various groups. Pesticides have been used since very ancient times.

Initially, pesticides had tremendous use in improving the quality of life and survival rate of humans by providing sufficient food. The overuse of chemical pesticides has harmful effects on the environment, ecosystems, human health, and mobility, causing toxicity to non-target species, including man also.Being aware of these damages, several measures have been taken, including the restricted use of some hazardous compounds and the practise of an eco-friendly approach to pest management.

Abhilash & Singh (2009) studied agricultural development opportunities as an important objective of Indian planning and policy. In the process of developing agriculture, pesticides have become a very important tool as a plant protection agent. Pesticides control many pests and diseases on plants, but exposure to pesticides causes environmental and human health problems. It has been observed that exposure to pesticides causes immune suppression, hormone disruption, reproductive abnormalities, and cancer. India is the largest producer of pesticides in Asia and ranks 12^{th} in the world for pesticide use.

Nag & Raikwar (2008) carried out monitoring in India to evaluate the status of organochlorine pesticide (OCP) residues. Out of a total of 325 samples, 20663.38% were contaminated with residues of different OCPs. Reed and coworkers (1987) described the problem of food contamination with pesticide residues as a concern for everyone. Most countries started routine examination of food to detect contamination levels and also ensure that the product does not have any pesticide residues that exceed its tolerance limit. The pesticide residues higher than the tolerance limits (maximum residue limits) in the crop cause great concern for the nation and the world. The serious problem occurs when improper, wasteful, and ultimately harmful applications of pesticides are applied to vegetables and other food commodities. Due to the presence of residues in

food, it is hazardous for human health, is exported to other countries, and also causes environmental pollution (FAO/WHO, Pesticide Residue in Food, 1989).

3. Objectives of the study:

1. To study the pesticide residue in major consuming leafy vegetables.
2. To study the phytochemical analysis of leafy vegetables.
3. Estimation of pesticide residues in leafy vegetables by GC-MS analysis method.
4. To determine the occurrence and concentration of multiclass pesticide residues in leafy vegetables.
5. To synthesize a botanical pesticides to control pests and diseases on leafy vegetables.

4. Materials and Methods:

4.1. Collection of Leafy vegetables:

A sample of leafy vegetables will be collected from a local markets in the Marthwada region. The collected samples will be packed in sterile polythene bags and these samples will be transported to the laboratory, where they will be analysed immediately or stored at 4°C and analysed within 24 hours.

4.2. Pesticide residue analysis by GC-MS method:

Pesticide residue analysis wil be done by the Gas chromatography equipped with Mass spectrography(GC-MS Analysis).

4.3.Extraction of samples:

A fresh sample of leafy vegetables will be taken for the extraction of pesticide residues. Each vegetable will be chopped into small pieces. A representative sample (20 g) will macerated in a blending machine to make a fine paste. The macerated sample will be extracted on a mechanical shaker for 1 hour.

4.4. Phytochemical Analysis:

phytochemical analysis will be carried out for identification of active chemical constituent like Alkaloids, Glycosides, Terpenoids, Flavonoids, Saponins, Tannis, Reducing sugar by following tests:

(i)Test for alkaloids:

(ii)Test for glycosides:

(iii)Test for Terpenoid:

(iv)Test for Flavonoid:

(v)Test for Tannis:

(vi)Test for saponin:

4.5. Bicontrol:

Biocontrol of pests & diseases carried out by using the Botanical pesticides.

4.6. Statistical analysis:

5. Importance of study:

National status:

The use of pesticides provides valuable profit for agricultural production. The discovery of pesticidal residues in various sections of the environment has raised concerns. Due to the pesticide residues, they not only cause human diseases but are also highly toxic to aquatic life. According to the joint report by WHO and UNEP, about 200,000 people die and 3 million are poisoned each year due to pesticides all over the world (WHO/UNEP, 1990; FAO/WHO;2000).

In India, pesticide use was banned in 1985 with the exemption of DDT, which is still used to control malaria (UNEP 2003). In India, the consumption pattern of pesticides is different from the rest of the world. In India, the rate of insecticide consumption is high, i.e., 76%, while herbicides are 10% and fungicides are 13%, respectively. This is in stark contrast to the rate of world consumption, i.e., herbicides at 30% and fungicides at 21%, which is higher. (Mathur et al.,1999). The Indian government banned the production, import, and use of 25 pesticides out of the total currently registered. while the government also adopted IPM as the dominant principal for plant protection to reduce chemical control. At present, 31 central IPM centres are operating across India to monitor pests and diseases and to produce biocontrol agents as an alternative to synthetic pesticides.

International status:

Approximately 2 million tonnes of pesticides are consumed worldwide each year, of which 24% are consumed in the USA, 45% in Europe, and the remaining 25% in the rest of the world. (Abhilash & Singh, 2009). In the most developed countries, i.e., North America,Western Europe, and Japan, the use of pesticides is high. They consume three-quarters of the total pesticides used worldwide. (USEPA 2009) . The quantity of pesticides consumed in developing nations is lower as compared to developed countries. & it's growing steadily. (Wilson & Tisdell, 2001; WRI, 1998). The international community has been greatly concerned about the health risks associated with pesticides and has sought mutual cooperation and action from all nations to reduce and eliminate the production of pesticides (Ishwar et al., 2015).

Significance of the study:

Pesticides used in agriculture to control pests and diseases have been subject to considerable legislative, regulatory, and consumer scrutiny. Over the past few decades, pesticides have become toxic in nature to living organisms. Many pesticide residues remain on foods available for human consumption. Due to the consumption of pesticide residues in food, serious problems occur for the public's health. The information on levels of pesticide residues remaining in food commodities is essential and can be obtained through regular monitoring by producers. The analytical surveys provide information regarding the use of certain persistent chemicals that may pose serious hazards to human health and the environment. The residue monitoring programmes create a database to help in assessing the levels of pesticide residue. Many countries in the world have established analytical laboratories to monitor residue levels in food. (Sharma et al., 2007). Due to pesticide pollution, environmental factors like the lives of birds, wild animals, domestic animals, and fish get affected; therefore, the determination of pesticide residues in food is necessary to protect the environment and human health.

6.Year wise plan of work:

1st year: * Review of literature.
* Collection of leafy vegetables.
* Preparation of extracts of leafy vegetables.

2nd year: * Pesticide residue analysis of leafy vegetables by GC-MS system.
* Study of Phytochemical analysis.
* Study of Antimicrobial activity.

3rd year: * Study of pest manegment by using botanicals.
* Preparation of Articles & papers.
* Submission of Thesis.

7. References

1. **Abhilash P.C. & Singh N (2009).** Pesticide use and application: an Indian scenario. *J. Hazardous Materials.*,165.

2. **Chanda I. (2014).** Pesticide: Use, abuse and awareness. *International Journal Current science*, 13:16-25.

3**. Ishwar Chandra Yadav (2015).** Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India Ishwar Chandra Yadav et al. *Sci Total Environ*.

4. **Damalas C. A. & Eleftherohorinos I.G. (2011).** Pesticide exposure, safety issues, and risk assessment indicators. *International Journal of Environmental Research and Public Health*, 8(5), 1402-1419.

5. **Ecobichon D.J. (2001).** Pesticide use in developing countries. *Toxicology*. 160:27-33.

6. **FAO (2017).** FAOSTAT, Crop data base. *Food and Agriculture Organization United Nations.* Availablefrom:http://www.fao.org/faostat/en/#data/QC/visualize.

7. **FAO/WHO (2002).** Global forum of food safety regulators. Marrakech, Morocco. [http://www.fao.org/DOCREP/MEETING/004/AB428E.HTMAgendaItem4.2a,GF/CRD Iran-1].

8. **FAO/WHO (2000).** Pesticide residues in food — 1999 evaluations. Part II — *toxicological*.Joint FAO/WHO Meeting on Pesticide Residues. World Health Organization, Geneva.

9**. FAO/WHO (1998).** Food Standards Program of Codex Alimentarius Commission about *pesticide residues in food*-Maximum residue limits. Vol. 2 B, Rome.

10. **Khandakar K. (1990).** Population, Agriculture And Environmental Degradation. An article published in the daily"*The Bangladesh Observer*".

11**. Koul O. & Walia S. (2009).** Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. *CAB Rev* 4(49):1–30 Available via *CABI.*

12. **Lv J, Wilson LT, Beuzelin JM, White WH, Reagan TE & Way MO. (2011).** Impact of Cotesiaflavipes (*Hymenoptera: Braconidae*) as an augmentative biocontrol agent for the sugarcane borer (*Lepidoptera: Crambidae*) on rice. *Biol Control* 56(2):159–169.

13. **Mathur S.C. (1999).** Future of Indian pesticides industry in next millennium. *Pestic. Inf*. 24(4), 9–23.

14. **Monitoring of Pesticide Residues at National LevelAnnual Progress Report(April, 2013 – March 2014).** Department of Agriculture and Cooperation Ministry of Agriculture Krishi Bhawan, New Delhi.

15. **Nag S.K. and Raikwar M.K. (2008).** Organochloride pesticide residues in bovine milk. *Bulletin of Environmental Contamination and Toxicology*, 80, 5-9.

16. **Reed D.V., Lombardo P., Wessel J.R., Burke J.A. and Mc Mohan B. (1987).**The FDA pesticides monitoring program. *J.AOAC*, 70:591-595.

17. **Kumbhar C R. (2020).** Role and mechanism of *botanicals in pest management* Kumbhar C R .

18. **Sharma K.K. (2007).** Directorate of Infomation and Publications of Agriculture, New Delhi. *Pesticide Residue Analytical Manual,* p: 291.

19. **Thacker JRM (2002).** An introduction to arthropod pest control.*Cambridge University Press*, Cambridge.

20. **Torres C.M., Pico Y. and Manes J.(1996).** Determination of pesticide residues in fruit and vegetables - a review. *J. Chromatogr. A.* 754. 301-331.

21. **Wilson C. & Tisdell C. (2001).** Why farmers continue to use pesticides despite environmental, health and sustainability costs. *Ecol. Econ.* 39, 449–462.

22**. UNEP (2003).** Global Report on Regionally Based Assessment of Persistent Toxic Substances. UNEP Chemicals, Switzerland, Geneva.

23.**USEPA (2009).** The Value of Country Working Together to Regulate *Pesticides and Food Safety* — Achieving Public Health and Environmental Protection Through International Collaboration.

24. **WHO/UNEP (1990).** Public Health Impact of Pesticides Used in Agriculture. *World Health Organization, Geneva.*

25. **Xiao YM, Wang JA, Wang MA, Liu JP, Yuan HZ, Qin ZH. (2010).** Study on the Inclusion Complexes of Flumorph and Dimethomorph with β-Cyclodextrin to improve fungicide formulation. *Journal of the Chemical Society of Pakistan* 32:363-369.

Signature	Signature
Research scholar	Supervisor